Functional and Evolutionary Implications of Opposed Bands, Big Mouths, and Extensive Oral Ciliation in Larval Opheliids and Echiurids (Annelida)

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Abstract. Larvae of two annelids, the opheliid *Armandia brevis* and the echiurid *Urechis caupo*, captured small particles between opposed prototrochal and metatrochal ciliary bands and also captured large particles with wide ciliated mouths. The body volume of larval *A. brevis* increased more rapidly than the estimated maximum clearance rate as segments were added. Capture of larger particles by late-stage larvae may compensate for this potentially unfavorable allometry. The existence of larvae that use two feeding mechanisms at once, not previously known in annelids, suggests possible evolutionary routes between larval forms that feed only with opposed bands (*e.g.,* serpulids and oweniids) and those that use complex oral ciliature to feed primarily on large particles (*e.g.,* polynoids and nephtyids). In particular, the metatroch and food groove of opposed-band feeders may have arisen as expansions of oral ciliation in ancestral large-particle feeders; alternatively, extensive oral ciliation in large-particle feeders may have originated as a modification of metatroch and food-groove cilia in ancestral opposed-band feeders.

Introduction

The trochophore is a larval form of several phyla: Annelida, Sipuncula, Mollusca, and Entoprocta (Nielsen, 1995). It is largely defined by the presence of the prototroch, a preoral ciliary band with a well-defined cell lineage. Despite this and other embryological similarities, trochophores are structurally and functionally diverse. Much of this diversity is found among the approximately 70 families of annelids in which larvae occur. Annelid larvae vary in the number and position of ciliary bands (though almost all possess a prototroch), and in whether or not they feed. Among annelid larvae that feed, mechanisms of capturing suspended particles have been described in only a few species (Strathmann, 1987).

One of these feeding mechanisms involves capturing and transporting particles with the prototroch and several postoral ciliary bands. The prototroch beats with an anterior-to-posterior effective stroke. A postoral band, the metatroch, parallels the prototroch and beats in opposition to it, with effective strokes from posterior to anterior. Particles small enough to fit between the prototroch and metatroch are captured between these two ciliary bands and transported to the mouth by a band of shorter cilia, the food groove. Particle capture by opposed bands has been described in larvae of two annelid families, the serpulids and the oweniids (Strathmann *et al.,* 1972; Emlet and Strathmann, 1994), and larvae of several other families possess the ciliary bands necessary to feed in this way.

Another feeding mechanism known in annelid larvae involves active responses to individual food particles. For example, polynoid larvae lack an opposing metatroch and a ciliated food groove. These larvae swim forward until they encounter relatively large particles, then manipulate each particle individually into the mouth with a tuft of long compound cilia (Phillips and Pernet, 1996). Larvae belong-

ing to related families (*e.g.*, phyllodocids and nephtyids: Rouse and Fauchald, 1997) also lack a metatroch and a food groove (Bhaud and Cazaux, 1987), and are able to capture particles as large as bivalve larvae, but how they do this is not known. Additional feeding mechanisms are known or suspected from larvae of other annelid families (Strathmann, 1987; Nielsen, 1998).

The structural and functional variety of trochophores in annelids and related phyla has raised questions about their evolution (Strathmann, 1993; McHugh and Rouse, 1998). There is no consensus as to whether feeding or nonfeeding larvae are ancestral, or on which feeding mechanisms are primitive (Strathmann and Eernisse, 1994). Given uncertainties about such key issues as the distribution of traits among clades, the functional requirements for capturing particles, and the phylogeny of annelids, inferences about ancestral character states are weak.

Our study describes ciliation and mechanisms of particle capture in larvae of two families of annelids, the Opheliidae and the Echiuridae. We use these observations to compare the feeding capabilities of different annelid larvae and to suggest possible evolutionary transitions among annelid larval forms. These data also augment the number of informative characters available for phylogenetic inferences.

Hermans (1978) showed that larvae of the opheliid *Armandia brevis* possess prototrochal and metatrochal ciliary bands. Although the feeding mechanism was not described, these observations suggest that opposed-band feeding may occur. He also noted that late-stage *A. brevis* larvae are able to ingest large particles. A larva with 15 segments had ingested a tintinnid 80 μm in diameter and a diatom 35 μm in diameter and 260 μm long (Hermans, 1964). This implies that these larvae were using a different feeding mechanism, since other work on annelid and mollusc larvae indicates that opposed-band feeding is limited to particles that fit between the prototroch and metatroch (typically spaced <30 μm apart: Strathmann *et al.*, 1972; Strathmann and Leise, 1979).

Thus, limited observations suggested that *A. brevis* larvae might use several feeding mechanisms to capture particles of a broad range of sizes. Alternative mechanisms for the capture of larger particles by later stage larvae might supplement the opposed-band feeding mechanism. Such versatility might be particularly advantageous to later stage larvae if unfavorable allometric relationships reduce the profitability of opposed-band feeding as development progresses. An unfavorable allometry might occur if body volume and metabolic demands increase more rapidly than ciliary band area and maximum clearance rates as segments are added during development. Therefore, in addition to observing particle captures, we examined the relationship between clearance rates and body volume.

Echiurids have sometimes been placed in the phylum Annelida and sometimes in their own phylum, the Echiura,

which is distinguished from the annelids by an apparent lack of segmentation (Nielsen, 1995). McHugh's (1997) molecular evidence shows that they are derived annelids, and she suggests that they should be placed in the annelid family Echiuridae. Larvae of the echiurid *Urechis caupo* bear prototrochal, metatrochal, and food-groove cilia (Newby, 1940; Suer, 1982), but how they capture particles has been unknown. We observed larval feeding in *U. caupo* to confirm use of the opposed-band feeding mechanism in the Echiuridae; to our surprise, we also obtained evidence that larger particles are captured at the mouth.

Our observations demonstrate that larvae in the annelid families Opheliidae and Echiuridae are able to capture particles both with opposed bands and directly at the mouth. This previously unrecognized combination of feeding mechanisms suggests hypotheses for evolutionary transitions among the diverse feeding larval forms of the Annelida.

Materials and Methods

Larval cultures

Reproductive adults of the opheliid *Armandia brevis* were collected in April and May 1998 in front of the Friday Harbor Laboratories, San Juan Island, Washington. Some animals were taken from beneath cobbles in the mid-intertidal zone and others from the plankton swarming at night to a light suspended from the laboratory dock. We isolated adults in finger bowls containing bag-filtered seawater (mesh size $\leq$ 10 μm) until gametes were released. Eggs were fertilized by the addition of sperm and then rinsed with filtered seawater. Fertilized eggs were placed in 450-ml beakers that held filtered seawater and were partially submerged in a seawater table at 11°–13°C. Larvae were fed a mixture of the algae *Isochrysis galbana* and *Chaetoceros gracilis*.

Adults of the echiurid *Urechis caupo* were dug in intertidal mudflats in Bodega Harbor, California, in June of 1995 and held in aquaria at the Bodega Marine Laboratory for use throughout the summer. Methods described by Gould (1967) were used for obtaining gametes and fertilizing eggs. We reared larvae in 800-ml beakers cooled in aquaria at 10° to 16°C (median 13.3°C), approximately the temperature of the coastal seawater. The seawater was filtered through meshes of 30 or 70 μm and larvae were fed the alga *Rhodomonas* sp. and occasionally *Isochrysis galbana* in addition to whatever food entered with the filtered seawater.

Ciliary bands

Light microscopy provided information about the ciliation of both opheliid and echiurid larvae. Larvae were viewed with differential interference contrast (DIC) optics

for an optical section through the prototroch, food groove, and metatroch.

Scanning electron microscopy provided additional information about the ciliation of *Armandia brevis*. Larvae were relaxed in a 1:1 mixture of 7.5% $MgCl_2$ and seawater for 30 min and fixed in 1% OsO_4 in seawater. After a rinse in seawater, fixed larvae were dehydrated in ethanol, infiltrated with hexamethyldisilazane for 30 min, and air-dried. They were mounted on stubs with double-sided tape and sputter-coated with gold-palladium before viewing.

Analysis of particle capture

To record larval feeding, we used video cameras mounted on compound and dissecting microscopes. A time-date generator indicated intervals between video images to the nearest 0.01 s. Larvae of *Armandia brevis* were presented with small and large particles in separate trials, and feeding activity was recorded at room temperature (22°C) onto VHS tape. We observed capture of small particles by placing several larvae on a slide with polystyrene-divinylbenzene spheres (Duke Scientific) of 5 and 12 μm diameter (one size per slide), adding a raised coverslip, and viewing the larvae with a 20× objective and DIC optics. Larvae that had tethered themselves with mucous strands and were actively feeding (indicated by beating of both the prototroch and

metatroch) were videotaped for about 10 min. We observed capture of large particles by placing larvae in a small petri dish onto a dissecting microscope and adding Sephadex beads ranging from 20 to 80 μm in diameter. Larvae were videotaped as they swam and fed.

For *Urechis caupo* larvae, feeding was observed at 15° to 20°C and recorded onto 8-mm tape. Larvae were confined within the spaces of a nylon mesh placed on a slide topped with a coverslip; they were free to rotate and change orientation but not to move forward continuously. We presented the larvae with three types of particles: the dinoflagellate *Prorocentrum micans* (length about 20 μm), polystyrene-divinylbenzene spheres (diameter 5 to 29 μm), and Sephadex beads (diameter 20 to 80 μm).

The size of particles captured and ingested by *U. caupo* was analyzed by inspecting the gut contents of particle-fed larvae. Larvae and suspensions of particles of several sizes were placed in vials that were rotated at 15 rpm. After 5 min, the larvae were fixed with formaldehyde for gut-content examination.

Scaling of clearance rate and body volume

The relationship of maximum clearance rate to body volume was estimated for larvae of *Armandia brevis* with 6 to 16 setigerous segments. We counted the number of seti-
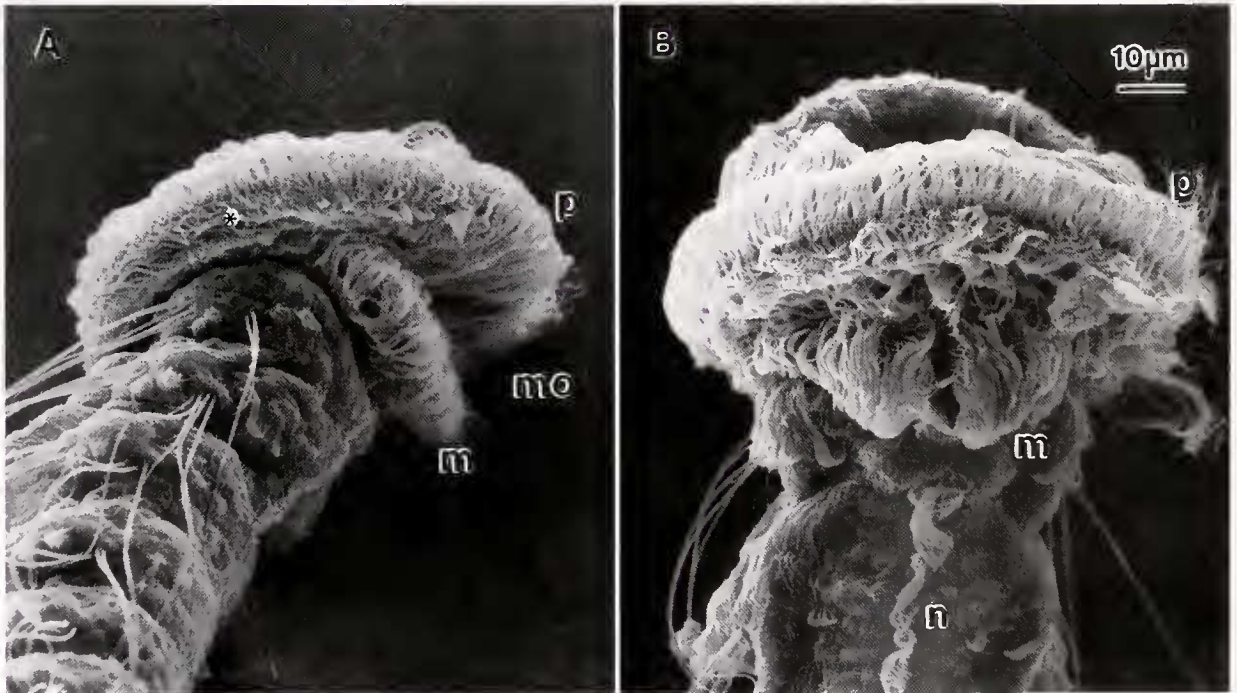

Figure 1. Scanning electron micrographs of larvae of *Armandia brevis*. (A) Posterolateral view of the anterior end of an 18-setiger larva. The food groove (*) is the region between the long compound cilia of the prototrochal (p) and metatrochal (m) ciliary bands. The inner surfaces of the mouth (mo) are heavily ciliated. (B) Ventral view of the anterior end of an 18-setiger larva, showing the long compound cilia of the metatroch (m) on the lower lip, the prototroch (p), and the short neurotroch (n). Both photos are to the same scale.

gers and measured body length (for the entire larva), width (at the middle segment), and prototroch diameter of live larvae ($n = 36$) under a compound microscope with 4× objective. A video camera and image analysis program (NIH Image 1.61; available free at http://rsb.info.nih.gov/ nih-image) were used for these measurements. We estimated larval volume as a cylinder by the equation:

$$\text{larval volume} = \pi(D/2)^2(L)$$

where D is body width and L is body length.

Maximum clearance rates were estimated as the volume of water passing through the prototroch per unit of time. To calculate these rates, we measured particle velocities and particle distances to the base of the prototroch from videotaped sequences of three larvae in each of three size classes (6–7, 11–12, and 15–16 setigers). We observed larvae and 5-μm particles on a compound microscope with DIC optics and 20× objective lens, as described above. The larvae tethered themselves by mucous strands and were recorded for several minutes. The distances traveled by particles per unit of time and their distances to the base of the prototroch were measured from videorecorded sequences. Particles were measured as they passed within the direct influence of the cilia where velocities are negligibly affected by the slide or coverslip (Emlet, 1990).

We fitted binomial regressions from the origin through the plot of particle velocity versus particle distance from the cilium base. The rationale for fitting curvilinear lines to these data was both theoretical (Sleigh, 1984) and empirical (Strathmann and Leise, 1979). The studies in both areas suggest that velocity should increase from zero near the larval body surface to a maximum near the full length of the cilia; it should then decrease beyond the tips of the cilia. Since these curves included some particles that presumably passed beyond the tips of the cilia, it was necessary to estimate the lengths of the cilia for larvae of each of the three size classes. We measured cilium lengths (15 cilia per larva) with NIH Image from videotaped, live larvae with 0–17 setigers ($n = 22$ larvae). The binomial regression equations relating particle velocity to particle distance from the cilium base were then integrated from the origin (the base of the prototroch) to the estimated cilium length for that size class. The resulting areas represent estimates of the area of water that, in one unit of time, passes through one optical section of the prototroch in the plane of ciliary beat.
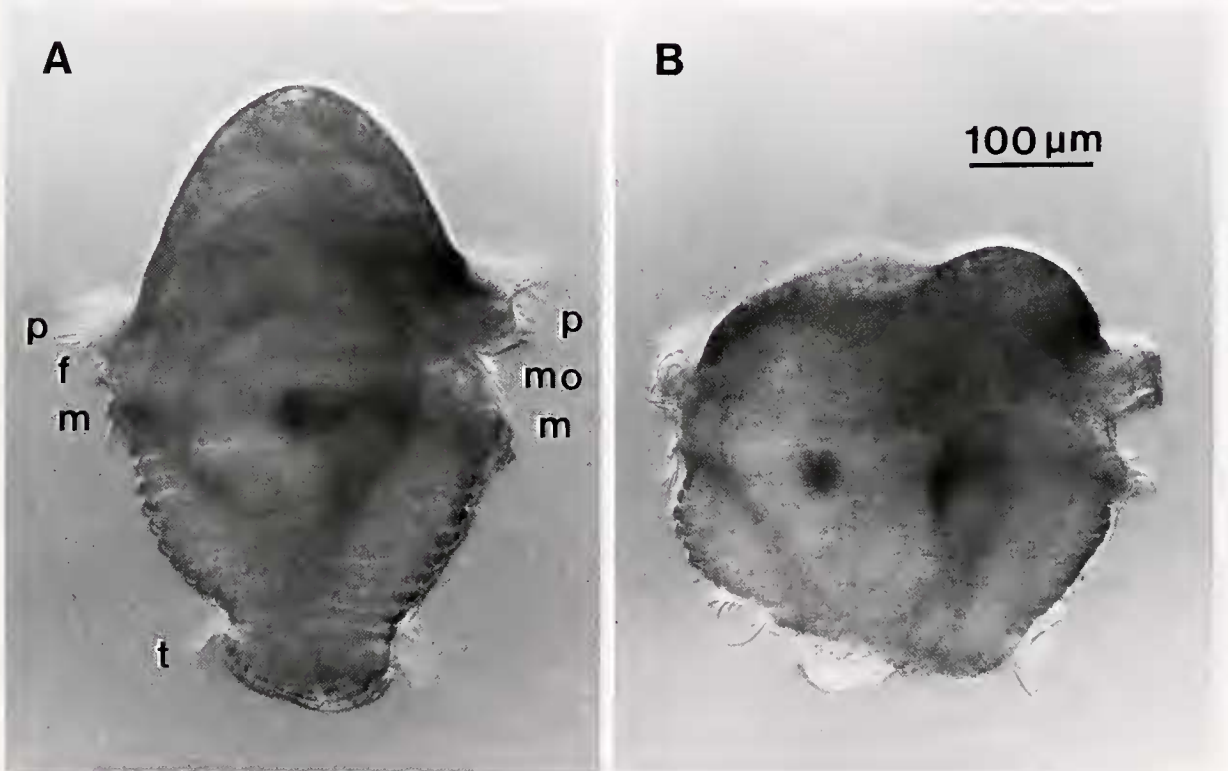

Figure 2. Light micrographs of larvae of *Urechis caupo.* (A) Lateral view with plane of focus through the prototroch (p), food groove (f), metatroch (m), and telotroch (t), dorsally. Ventrally, the plane of focus passes through the prototroch (p), mouth (mo), and metatroch (m). The neurotroch is not visible. The dark spot near the center is a particle in the gut. (B) The same larva when contracted. Both photos are to the same scale.

We then estimated maximum clearance rates for each size class by multiplying that value by the circumference of the prototroch halfway between the base of the cilium and its tip (midpoint prototroch circumference). Finally, to determine whether maximum clearance rate scaled proportionately to body size during larval growth, we divided the maximum clearance rate for a given size class by the average body volume for that size class.

Results

Ciliary bands

Scanning electron micrographs clearly show the prototrochal and metatrochal cilia of *Armandia brevis*. The prototroch is made up of several rows of compound cilia that completely encircle the larval body anterior to the mouth (Fig. 1). The metatroch is a postoral band of compound cilia that extends laterally from the lower lip of the mouth around the larval body to a dorsal position (Fig. 1A). The metatrochal cilia on the lower lip are longer than the other metatrochal cilia (Fig. 1A, B). The prototroch and metatroch define the boundaries of a cilia-lined food groove. The width of the food groove lateral to the mouth was estimated from a scanning electron micrograph to be 10 μm (SEM not shown). Dorsally, the food groove narrows. The mouth is large (about 50 μm wide in the 18-setiger larva shown in Fig. 1B) and both its upper and lower surfaces are heavily ciliated (Fig. 1A, B). A band of neurotrochal cilia runs along the ventral surface of the larva from just behind the mouth to the third setigerous segment (Fig. 1B).

Larvae of *Urechis caupo* also possess prototrochal and metatrochal ciliary bands (Fig. 2). The prototrochal cilia are longer than the metatrochal cilia. Again, these two ciliary bands define the boundaries of a food groove lined with simple cilia. Larvae also bear a midventral neurotroch, posterior to the mouth, and a telotroch.
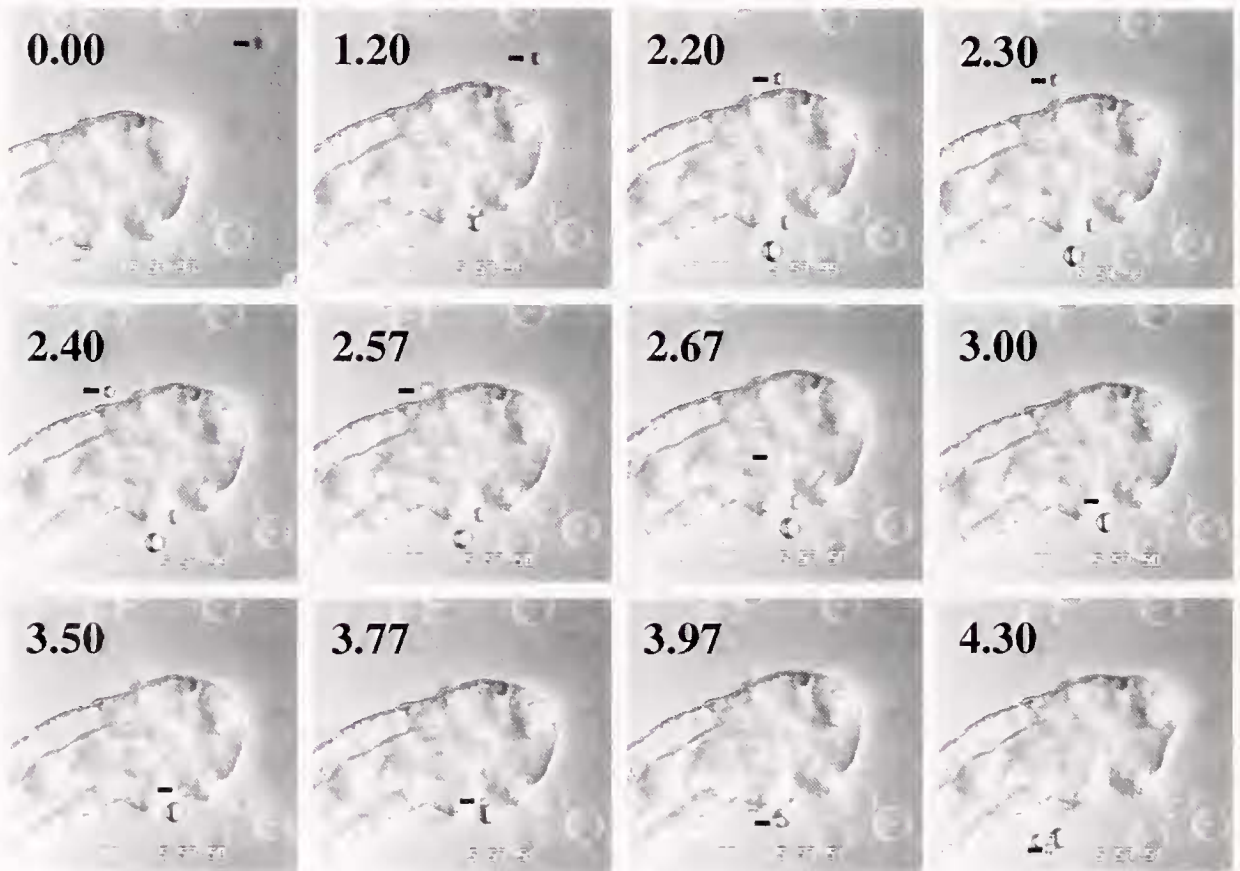

Figure 3. Videorecorded capture of a 5-μm sphere by opposed bands, and particle rejection by a self-tethered *Armandia brevis* larva. Time in seconds is in the upper left-hand corner. All images are at the same magnification. The larva is oriented with its dorsal side toward the top of the page. The sequence shows a particle, indicated by the black line, approach the dorsal part of the metatroch where it is captured and then transported along the food groove and deposited in the mouth. The particle is then rejected. During rejection the metatroch on the lower lip ceases to beat. At 0 s the larva is 120 μm in diameter at the base of the prototrochal cilia.
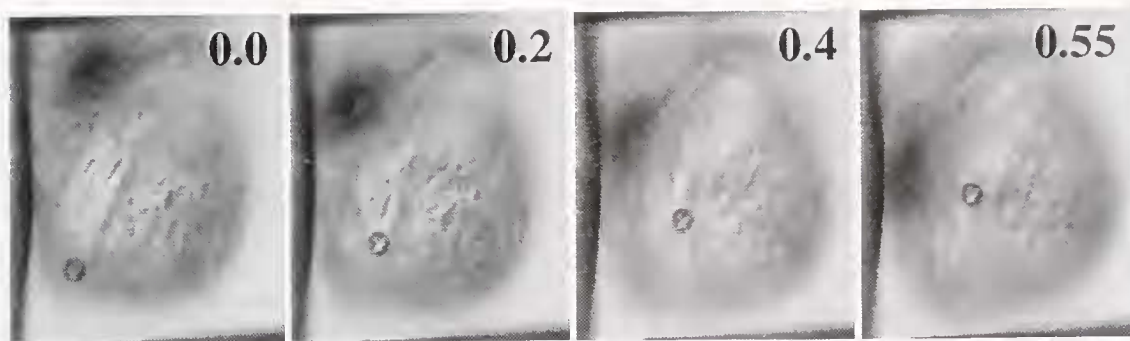
Figure 4. Videorecorded capture of a 13-μm sphere by a *Urechis caupo* larva. Time is in seconds in the upper right-hand corner. The particle has entered a dorsolateral part of the food groove at 0 s, moves along the food groove toward the mouth at 0.2 and 0.4 s, and enters the side of the mouth at 0.55 s. Rotation of the larva moves the mouth from upper right at 0 s to center at 0.55 s. The anterior end of the larva is toward the upper left. At 0 s the larva is 175-μm wide at the base of the prototrochal cilia.

Capture by opposed ciliary bands

In larvae of *Armandia brevis* and *Urechis caupo*, the movements of particles and the directions of recovery strokes of cilia indicated that the effective strokes of the prototrochal cilia were from anterior to posterior, and those of the metatrochal cilia were from posterior to anterior. For larvae of each species, we observed captures of more than 50 particles of 5 and 12 μm in diameter; particles that came within reach of the prototroch were transported into the food groove between the prototroch and metatroch and moved to the mouth *via* the food groove, presumably by the food-groove cilia (Figs. 3–5). Particles were captured between prototroch and metatroch on the lateral and dorsal surfaces of the larva. Particles in the food groove moved around to the mouth from both the left and the right sides and both with and against the direction of rotation of the larval body. These particle paths indicate an opposed-band feeding mechanism.

Capture of large particles

Larvae of both species also captured particles at the mouth, without transport in the food groove. A late-stage larva of *Armandia brevis* (with > 14 setigers) captured two large particles (50 μm in diameter) while videorecorded through a dissecting microscope (Fig. 6). When the swimming larva contacted a large particle in the vicinity of the mouth, the larva slowed and rotated so that the lower lip was aligned with the particle. The larva opened its mouth and ingested the particle, presumably using oral cilia or musculature.

Swimming *Urechis caupo* larvae used the mouth for direct capture of particles that passed over the episphere. Such captures occurred simultaneously with opposed-band particle captures (Fig. 5). Many of the particles caught directly by the mouth were too large to fit between opposed prototroch and metatroch, as illustrated by the gut contents

in Figure 7 and the particles being rejected in Figure 8. In some cases the mouth gaped to admit a large particle. The 9-day-old larva in Figure 4 opened its mouth to a gape of about 35 μm with a width of 95 μm. The 17-day-old larva in Figure 8 opened its mouth to 70 to 95 μm, and the mouth's width when closed was about 125 μm. Cilia on the mouth's lower lip (anterior to the shorter cilia of the neurotroch) appeared to aid the movement of large particles into the mouth. These cilia seemed to be continuous with the metatrochal band, which would account for the posterior-
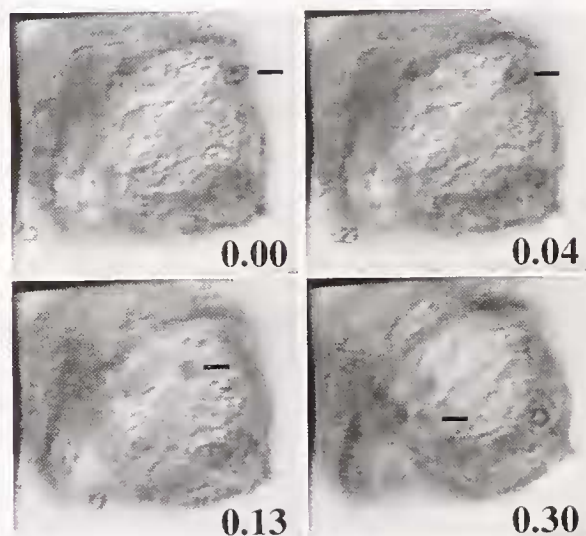

Figure 5. Videorecorded capture of two 12-μm spheres by a *Urechis caupo* larva. Time is in seconds in the lower right-hand corner. The particle marked by an adjacent black bar has entered a dorsolateral part of the food groove at 0 s, moves along the food groove toward the mouth at 0.04 and 0.13 s, and is near the side of the mouth at 0.30 s. The second particle passes over the prototroch directly into the mouth. It is near the prototrochal cilia at 0 s, passes over the anterior edge of the mouth at 0.04 and 0.13 s, and has entered the mouth at 0.30 s. The mouth is at the lower left; the anterior end toward the upper left. At 0 s the larva is 170-μm wide at the base of the prototrochal cilia.
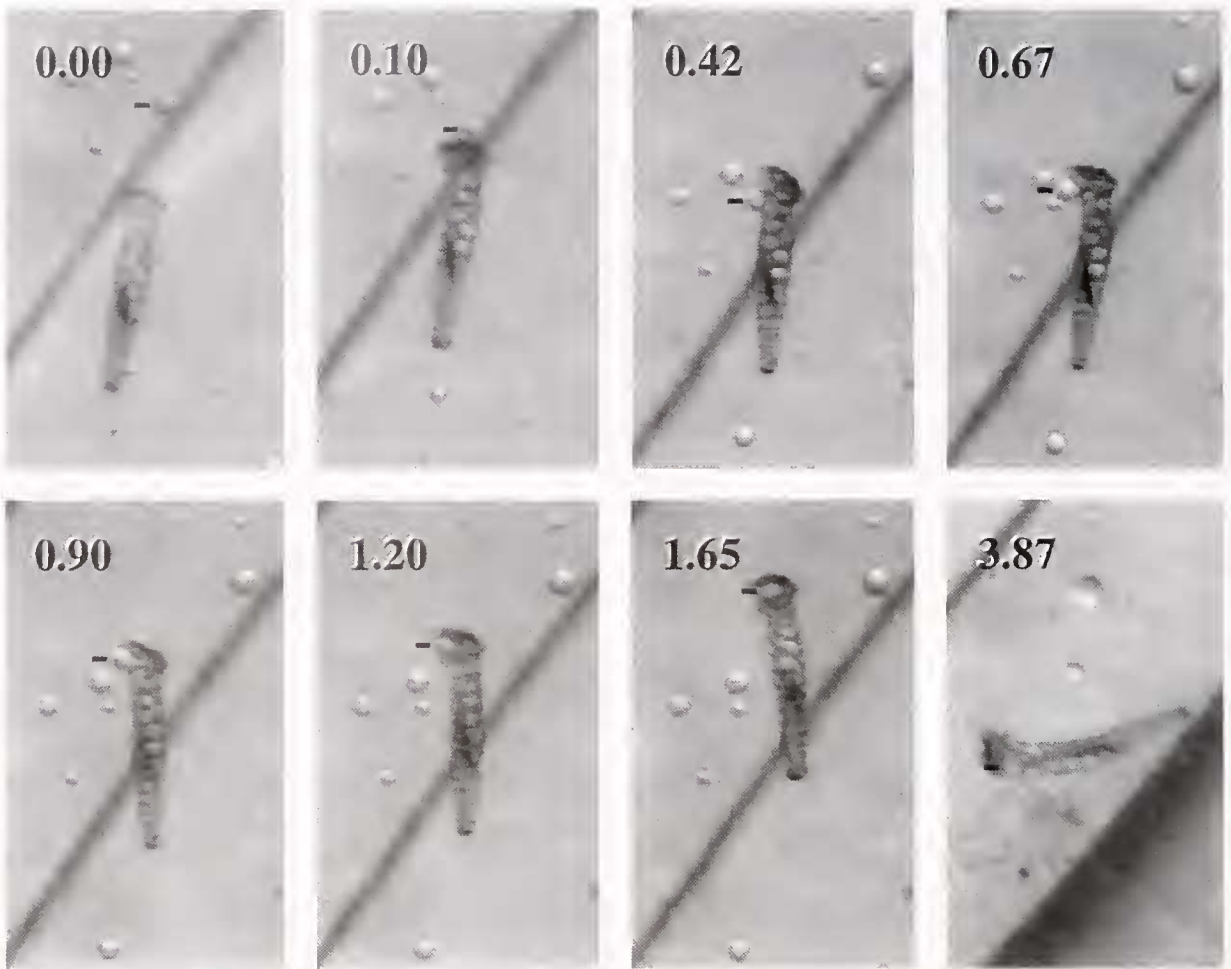
Figure 6. Videorecorded capture of a 50-μm sphere by a free swimming *Armandia brevis* larva under a dissecting microscope. Time in seconds is in the upper left-hand corner. All images are at the same magnification. A black line indicates the particle. The larva approaches the particle and then orients its mouth towards the particle, which is on the bottom of the dish. The particle is captured at the larva's mouth, presumably moved by the large oral compound cilia, and swallowed. At 0 s the larva is 85-μm wide at the center of the body.

to-anterior current past these cilia. In some cases a particle was brought into the mouth over the lower lip (Fig. 7).

Larvae of *U. caupo* captured large particles from an early stage. Small 4-day-old larvae ingested Sephadex spheres almost as large as those ingested by 16-day-old larvae (Table 1). Even a 3-day-old larva ingested a 42-by-35-μm mineral grain. Larger larvae did capture larger spheres, however. When early and later stage larvae were fed the same suspension, as in the last two lines of Table 1, the median sizes and the largest sizes of ingested spheres were significantly greater for larger, older larvae (Mann-Whitney *U* tests, $n_1 = 10$, $n_2 = 5$, $P < 0.05$). Objects larger than the spheres offered can be ingested. For example, a 49-day-old larva, 375 μm wide, ingested an unidentified object 366 μm long by 40 μm wide.

When larvae of *U. caupo* of different ages and sizes were offered smaller plastic spheres, all 10 of the small, 3-day-

old larvae caught fewer spheres of 29-μm than of 12-μm, and all 4 of the larger, 48-day-old larvae ingested more of the 29-μm spheres than of the 12-μm spheres (Table II). Small, early-stage larvae did ingest 5- and 20-μm spheres in about the same ratio as ingested by larger larvae (Table II). Estimates of the width of the food groove of a single 5-day-old larva ranged from 22 to 34 μm, but the width of the food groove varies with contraction of the larva. The upper limit on the sizes of particles that could be transported in the food groove was not determined.

Rejection of particles

Larvae could actively reject particles. Particle rejection often occurred after a particle had been transported to the mouth and entered the esophagus. When a larva of *Armandia brevis* expelled a particle, the metatrochal cilia around
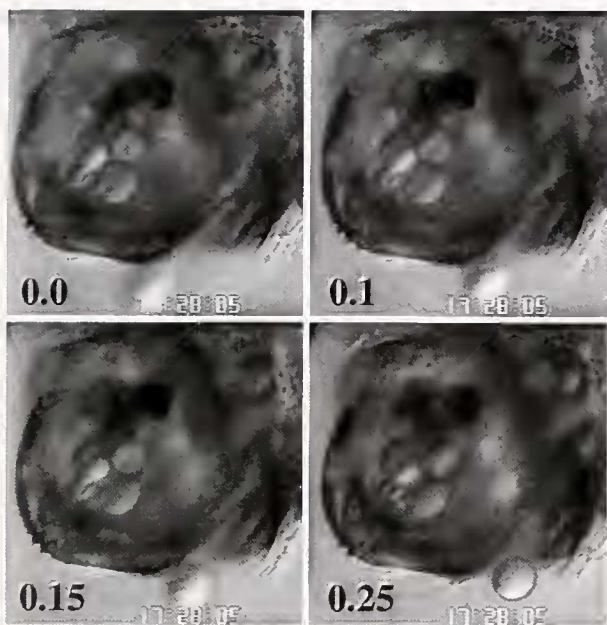
Figure 7. Videorecorded capture of a 40-μm sphere by a *Urechis caupo* larva. Time is in seconds in the lower left-hand corner. The sphere is near the metatrochal cilia at the posterior lip of the mouth at 0 s and moves over this band of cilia toward the mouth at 0.1 and 0.15 s. It is just entering the mouth at 0.25 s. The anterior end is toward the upper right. At 0 s the larva is 300-μm wide at the base of the prototrochal cilia.

the mouth stopped beating as the particle moved posteriorly down the body (Fig. 3). Metatrochal cilia at the mouth of larvae of *Urechis caupo* must also have altered beat during particle rejection, because large particles moved posteriorly over the lower lip and down the neurotroch during rejection (Fig. 8), in contrast to their posterior-to-anterior path over the lip during ingestion (Fig. 7).

Scaling of clearance rate and body volume

For larvae of *Armandia brevis*, prototroch circumference and prototrochal cilium length increased with number of setigerous segments (Fig. 9A, B). Larval volume increased exponentially with number of setigers (Fig. 9C).

Particle velocities increased slightly with number of setigers for larvae of *A. brevis* with 6–7, 11–12, and 15–16 setigers ($n = 9$) (Fig. 10). Increased particle velocities and cilium lengths resulted in a 30% increase in the area of water per prototrochal slice moved per second between larvae with 6–7 and 11–12 setigers and a 22% increase between larvae with 11–12 and 15–16 setigers (Table III). Maximum particle velocities were within the distal third of the cilium length (estimated for each size class from Fig. 9B), consistent with our expectations (Emlet and Strathmann, 1994). Although Strathmann *et al.* (1993) suggested that cilium lengths might be underestimated from videorecordings, our results indicate that this was not the case. In addition, our measurements agree with the cilium length of approximately 35 μm reported by Hermans (1964) for a larva with an unspecified number of setigers.

Although estimated maximum clearance rates increased with number of setigers, they did not increase proportionately to body volume (Table III). Late-stage larvae (15–16 setigers) had a maximum ratio of clearance to body volume that was less than half of that achieved earlier in development (6–7 setigers; Table III).

Prototrochal circumference and cilium length both increased with larval growth to a greater extent for larvae of *U. caupo* than for larvae of *A. brevis*, over the stages measured (Tables I–III). The relative increase in body length was much less for *U. caupo*. Early-stage larvae were nearly spherical and elongated to the shape shown in Figure 2A at later stages. Data for particle velocities are lacking for
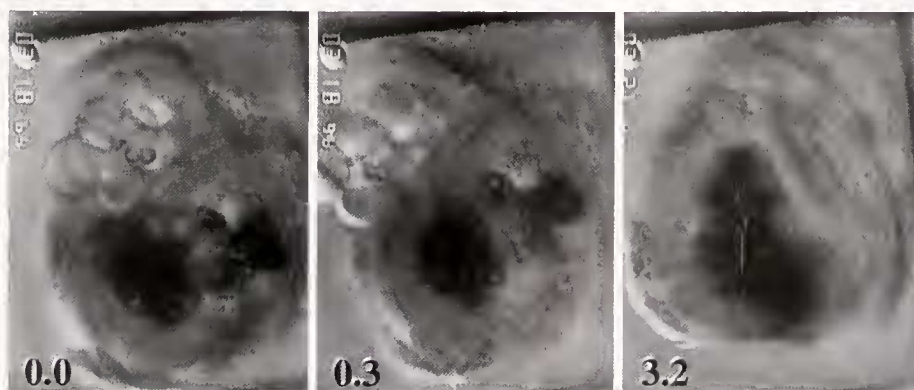

Figure 8. Videorecorded rejection of previously ingested spheres up to 50 μm in diameter by a *Urechis caupo* larva. Time is in seconds in the lower left-hand corner. At 0 and 0.3 s the mouth gapes at least 100-μm wide, and the clump of spheres moves over the posterior lip of the mouth and down the midventral neurotroch. The larva in the last frame is 295-μm wide at the base of the prototrochal cilia, and the mouth, now rotated toward the viewer, is closed and approximately 120-μm wide.

Table I

Sizes of Sephadex spheres ingested by larvae of Urechis caupo *differing in size and age*

Age (days)	Prototrochal diameter (μm)*	Cilium length (μm)	Particle diameter (μm)†		Number of larvae
			In suspension	Ingested	
4	159	45	45, 26–73 (50)	36, 19–53 (51)	12
5	165	44	44, 30–74 (50)	36, 19–60 (34)	10
16	318	65	44, 30–74 (50)	38, 21–73 (104)	5

* Diameter of the prototrochal band is diameter at the base of the prototrochal cilia.
† Values are median, range, and (in parentheses) number of particles.

U. caupo, but the increase in prototrochal area (cilium length times prototrochal circumference) relative to body volume was greater for this species than for *A. brevis.*

Discussion

Our observations add the Opheliidae and Echiuridae to those annelid families known to possess larvae with opposed-band feeding. As in other opposed-band feeders, larvae of both *Armandia brevis* and *Urechis caupo* possess a ciliated food groove between two parallel ciliary bands, a postoral metatroch and a prototroch. Direct observations confirm that particles are captured in the food groove (Figs. 3–5), probably through the combined action of long compound cilia in the prototroch (which beat anterior to posterior) and shorter compound cilia in the metatroch (which beat posterior to anterior). Simple cilia of the food groove may aid in retention of particles as well as in transport. This system is very effective in capturing relatively small particles (5–12 μm), regardless of which part of the prototrochal circumference is contacted (ventral, lateral, or dorsal). How common this feeding method is in larvae of other opheliids or echiurids is not known, but larvae of at least one other echiurid bear opposed bands of cilia (Salensky, 1876; Hatschek, 1880).

Larvae of both *A. brevis* and *U. caupo* also ingested particles larger than the space between prototrochal and metatrochal bands. For *A. brevis,* it was later stage (14–17 setiger) larvae that ingested large (50-μm) particles. These larvae approached large particles so that contact was directly at the mouth. This behavior was not observed in larvae at earlier stages. In contrast, larvae of *U. caupo* ingested particles greater than 50 μm at early stages. Larvae of *U. caupo* did not appear to change orientation as they approached large particles; however, their movements were constrained by mesh cages. Particles that were captured directly at the mouth entered either over the episphere and prototroch or over the extension of the metatroch on the lower lip. In both species the mouths were large, could be opened to a wide gape, and were heavily ciliated. The cilia bordering the lower lip of the mouth appear to be a continuation of the metatroch. The oral cilia of *A. brevis* may include additional compound cilia (Fig. 1). For both *A. brevis* and *U. caupo,* the large ciliated oral field and the large mouth aid in the capture of large particles.

The combination of two ciliary feeding mechanisms in individual larvae suggests hypotheses for evolutionary transitions among the feeding larvae of annelids. Some larvae, such as those of serpulids, appear to be restricted to capturing small particles between opposed bands; other larvae, like those of polynoids, lack opposed bands and appear to capture mostly large particles one by one, using complex oral ciliature (Phillips and Pernet, 1996). Our results dem-

Table II

Sizes of plastic spheres ingested by larvae of Urechis caupo *differing in size and age*

Age (days)	Prototrochal diameter (μm)*	Cilium length (μm)	Particle diameter		Number of larvae
			Ratio in suspension (29:12 μm)	Ratio ingested (29:12 μm)	
3	151	46	1.43:1	39/146 = 0.27	10
48	347	76	1.43:1	206/112 = 1.84	4
			(20:5 μm)	(20:5 μm)	
4	161	45	1:1	146/30 = 4.9	8
15	310	67	1:1	99/37 = 2.7	8

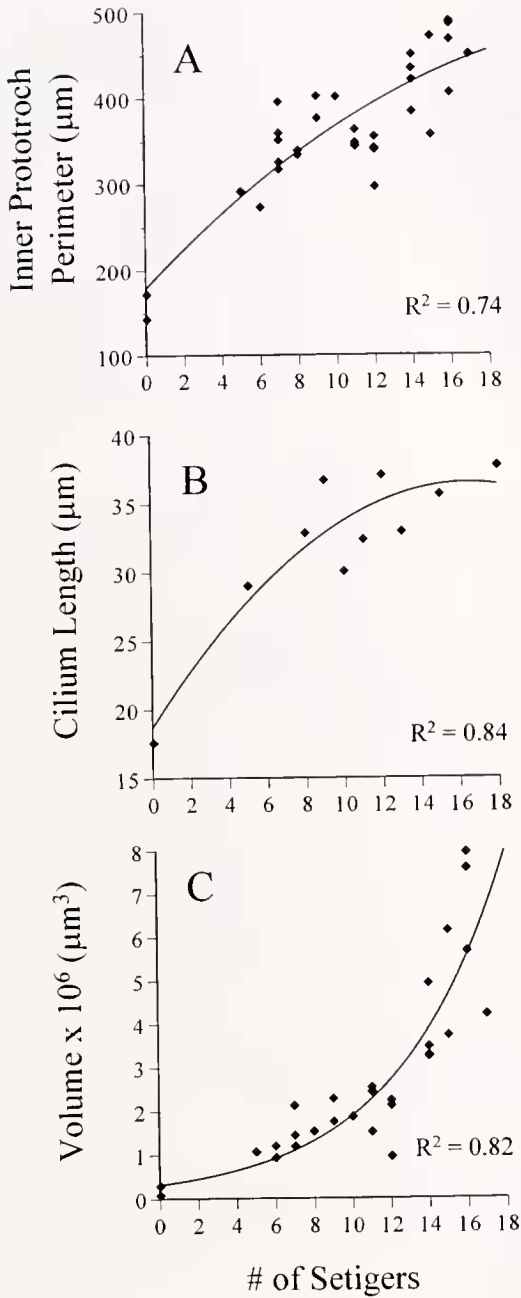* Diameter of the prototrochal band is diameter at the base of the prototrochal cilia.

extensions of the metatroch and food groove. As an evolutionary transition, expansion of oral ciliation might result in a food groove and metatroch paralleling the whole length of the prototroch to produce an opposed-band system. Alternatively, enlargement of the mouth and elaboration of oral ciliation (with loss of the lateral and dorsal parts of the opposed-band system) could produce the variety of oral
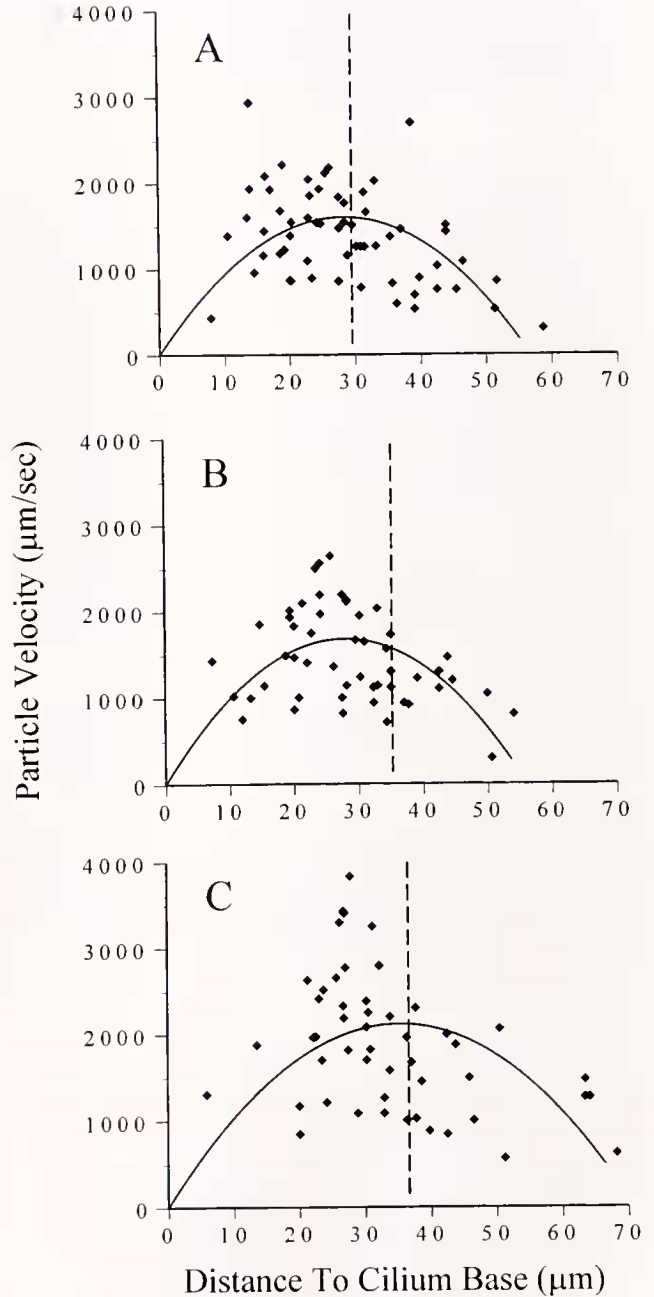

Figure 9. Binomial regression of various larval parameters *vs.* number of setigers for *Armandia brevis*. For all equations, X = number of setigers. The R^2 value is reported in the lower right-hand corner of each plot. (A) Inner prototroch perimeter (n = 36 larvae); larval circumference = $178.36 + 23.24x - 0.44x^2$. (B) Cilium length ($n$ = 22 larvae); cilium length = $18.67 + 2.16x - 0.07x^2$. (C) Larval volume (n = 36 larvae); larval volume = $10^{5.48 + 0.08x}$.

onstrate that in at least two families of annelids, both types of mechanisms can be employed simultaneously by the same larva. In addition, it appears that the oral ciliature of *A. brevis* and *U. caupo*, which is responsible for the capture of large particles, is continuous with the lateral and dorsal

Figure 10. Particle velocity *vs.* distance of particle from the base of the prototroch for *Armandia brevis* larvae with (A) 6–7, (B) 11–12, and (C) 15–16 setigers. The vertical dotted line shows the estimated cilium length taken from the binomial regression of Figure 9B.

Table III

Estimated clearance rate and clearance rate per larval volume for three size classes of larvae of Armandia brevis

# of Setigers	Cilium length (μm)[*]	Water area per prototroch slice per unit time[†] (μm²/s)	Midpoint prototrochal circumference (μm)	Max. clearance rate (μm³/s)× 10⁶	Larval volume (μm³)[‡]	Clearance rate/volume (1/s)
6–7	29.9	32846	422	13.9	998309	13.9
11–12	34.8	42602	549	23.4	2526475	9.3
15–16	36.4	51949	642	33.4	5310250	6.3

* Calculated from the binomial regression in Fig. 10B.

† Calculated from the areas under the curves in Fig. 11, bound by the origin and the estimated cilium length for that size class.

‡ Estimated from the binomial regression in Fig. 10C.

ciliature found in the diverse feeding larvae of annelids. Continued modification of such cilia might result in such unusual and functionally important structures as the group of long compound cilia on the left side of the mouth of polynoid larvae.

Estimated maximum clearance rates did not scale isometrically with body volume among the three size classes of *A. brevis*. Cilium length, prototroch circumference, and particle velocities through a prototrochal slice all increased as body volume increased, but not enough for maximum clearance rate to increase in proportion to body volume—thus the volume of water swept by cilia decreases relative to body volume as the larva adds segments. An analogous situation has been described for the cyphonautes larva of bryozoans, in which ciliated band length does not increase proportionately to body volume during growth and development (McEdward and Strathmann, 1987). This allometry is potentially unfavorable to larger larvae. In asteroid, echinoid, and bivalve larvae similar in size to *A. brevis* larvae, metabolic rates scale isometrically with body mass (Hoegh-Guldberg and Manahan, 1995). Further, in the larvae of an echinoid, metabolic demand scales isometrically with larval volume (McEdward, 1984). If these results can be generalized to larvae of *A. brevis*, and if we make the reasonable assumption that the masses of these larvae are proportional to their volume, then the maximum clearance rates of *A. brevis* larvae decline relative to metabolic demand as the larvae increase in size. However, larger larvae of *A. brevis* (>12 setigers) can supplement the amount of small particles captured by opposed-band feeding by capturing larger particles at the mouth. The increased size range of food may compensate, at least partly, for the decrease in clearance rate. This decrease in maximum clearance rate per larval volume may have selected for larvae that possess two types of feeding mechanisms.

Do other annelid larvae share this potentially unfavorable allometry of maximum clearance rate and body volume? Some annelid larvae resemble *A. brevis* in extreme elongation of a segmented body during the larval stage (Bhaud and Cazaux, 1987). Some of these larvae (*e.g.*, spionids) possess feeding mechanisms other than the opposed prototrochal and metatrochal bands. Thus, evolutionary changes in the size range of particles captured may have been favored in several groups of annelids as a result of a small head circumference and long larval body. Other possible solutions to this problem are opposed bands elongated on ciliated lobes, as reported for the rostraria larva of an annelid (Jägersten, 1972), or the sinuous opposed bands of mitraria larvae of oweniid annelids (Emlet and Strathmann, 1994).

The larvae of *U. caupo* and some other annelids probably do not face such an unfavorable allometry of maximum clearance rate to body volume, however. The larvae of *U. caupo* develop from nearly spherical trochophores (at 3 to 5 days) to forms with more elongate bodies (at several weeks), but the elongation is not as extreme (*cf.* Fig. 2 to Fig. 6). Also, these larvae capture relatively large particles from an early stage. Nevertheless, the circumferential ciliary bands are shorter, relative to body size, than similar bands that are extended on the velar lobes of many gastropod larvae (Richter and Thorson, 1975). Feeding on an extended size range of particles and extension of opposed, ciliary bands on lobes may be alternative ways of increasing ingestion rates.

Further analyses of larval feeding methods, as well as robust phylogenies, are required to understand the evolution and functional consequences of diverse larval feeding mechanisms in the Annelida. For example, why are opposed bands apparently used only in the capture of small particles? What functional constraints place an upper limit on the spacing of the prototroch and metatroch in opposed-band feeders? Such analyses may also reveal why some larvae (*e.g.*, serpulids) use restricted opposed bands to feed on small particles, and others (*e.g.*, polynoids) use complex oral ciliature to feed primarily on large particles instead of employing both methods, as do the opheliid and echiurid larvae described here.

Acknowledgments

Literature Cited

Bhaud, M., and C. Cazaux. 1987. Description and identification of polychaete larvae; their implications in current biological problems. *Oceanis* 13: 595–753.

Emlet, R. B. 1990. Flow fields around ciliated larvae: the effects of natural and artificial tethers. *Mar. Ecol. Prog. Ser.* 63: 211–225.

Emlet, R. B., and R. R. Strathmann. 1994. Functional consequences of simple cilia in the mitraria of oweniids (an anomalous larva of an anomalous polychaete) and comparisons with other larvae. Pp. 143–157 in *Reproduction and Development of Marine Invertebrates*, W. H Wilson, Jr., S. A. Stricker, and G. L. Shinn, eds. Johns Hopkins University Press, Baltimore.

Gould, M. 1967. Echiurid worms: *Urechis*. Pp. 163–171 in *Methods in Developmental Biology*, F. H. Wilt and N. Wessels, eds. Crowell, New York.

Hatschek, B. 1880. Über die Entwicklungsgeschichte von *Echiurus* und die systematische Stellung der Echiuridae. *Arbeiten Zool. Inst. Wien* 3: 45–78, plates 1–6.

Hermans, C. O. 1964. *The Reproductive and Developmental Biology of the Opheliid Polychaete*, Armandia brevis. M. S. Thesis, University of Washington. 131 pp.

Hermans, C. O. 1978. Metamorphosis in the opheliid polychaete *Armandia brevis*. Pp. 113–126 in *Settlement and Metamorphosis of Marine Invertebrate Larvae*, F.-S. Chia and M. E. R. Rice, eds. Elsevier, New York.

Hoegh-Guldberg, O., and D. T. Manahan. 1995. Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. *J. Exp. Biol.* 198: 19–30.

Jägersten, G. 1972. *Evolution of the Metazoan Life Cycle*. Academic Press, New York. 282 pp.

McEdward, L. R. 1984. Morphometric and metabolic analyses of the growth and form of an echinopluteus. *J. Exp. Mar. Biol. Ecol.* 82: 259–287.

McEdward, L. R., and R. R. Strathmann. 1987. The body plan of the cyphonautes larva of bryozoans prevents high clearance rates: comparisons with the pluteus and a growth model. *Biol. Bull.* 172: 30–45.

McHugh, D. 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proc. Natl. Acad. Sci. USA* 94: 8006–8009.

McHugh, D., and G. W. Rouse. 1998. Life history evolution of marine invertebrates: new views from phylogenetic systematics. *Trends Ecol. Evol.* 13: 182–186.

Newby, W. W. 1940. *The Embryology of the Echiuroid Worm* Urechis caupo. American Philosophical Society, Philadelphia. 219 pp.

Nielsen, C. 1995. *Animal Evolution*. Oxford University Press, Oxford. 467 pp.

Nielsen, C. 1998. Origin and evolution of animal life cycles. *Biol. Rev.* 73: 125–155.

Phillips, N. E., and B. Pernet. 1996. Capture of large particles by suspension-feeding scaleworm larvae (Polychaeta: Polynoidae). *Biol. Bull.* 191: 199–208.

Richter, G., and G. Thorson. 1975. Pelagische Prosobranchier-Larven des Golfes von Neapel. *Ophelia* 13: 109–185.

Rouse, G. W., and K. Fauchald. 1997. Cladistics and polychaetes. *Zool. Scr.* 26: 139–204.

Salensky, W. 1876. Über die Metamorphose des *Echiurus*. *Morphologisches Jahrbuch* 2: 319–327.

Sleigh, M. A. 1984. The integrated activity of cilia: function and coordination. *J. Protozool.* 31: 16–21.

Strathmann, R. R. 1987. Larval feeding. Pp. 465–550 in *Reproduction of Marine Invertebrates, Vol. 9. General Aspects: Seeking Unity in Diversity*, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell, Palo Alto, CA.

Strathmann, R. R. 1993. Hypotheses on the origins of marine larvae. *Annu. Rev. Ecol. Syst.* 24: 89–117.

Strathmann, R. R., and D. J. Eernisse. 1994. What molecular phylogenies tell us about the evolution of larval forms. *Am. Zool.* 34: 502–512.

Strathmann, R. R., and E. Leise. 1979. On feeding mechanisms and clearance rates of molluscan veligers. *Biol. Bull.* 157: 524–535.

Strathmann, R. R., T. L. Jahn, and J. R. C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull.* 142: 505–519.

Strathmann, R. R., L. Fenaux, A. T. Sewell, and M. F. Strathmann. 1993. Abundance of food affects relative size of larval and postlarval structures of a molluscan veliger. *Biol. Bull.* 185: 232–239.

Suer, A. L. 1982. *Larval Settlement, Growth, and Reproduction of the Marine Echiuran* Urechis caupo. Ph.D. Thesis, University of California, Davis. 198 pp.